ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2007, p. 23–27 Vol. 51, No. 1
0066-4804/07/$08.00+0 doi:10.1128/AAC.00788-06

Synergistic Activities of Azithromycin and Amphotericin B against *Naegleria fowleri* In Vitro and in a Mouse Model of Primary Amebic Meningoencephalitis[▽]

Shannon M. Soltow* and George M. Brenner

Department of Biomedical Sciences, Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma

Received 29 June 2006/Returned for modification 7 August 2006/Accepted 16 October 2006

***Naegleria fowleri* is responsible for producing a rapidly fatal central nervous system infection known as primary amebic meningoencephalitis (PAM). To date, amphotericin B, an antifungal agent, is the only agent with established clinical efficacy in the treatment of PAM. However, amphotericin B is not always successful in treating PAM and is associated with severe adverse effects. We previously found azithromycin to be more effective than amphotericin B in a mouse model of PAM. We therefore investigated the combination of amphotericin B and azithromycin in vitro and in a mouse model of PAM. For the in vitro studies, 50% inhibitory concentrations were calculated for each drug alone and for the drugs in fixed combination ratios of 1:1, 3:1, and 1:3. We found amphotericin B and azithromycin to be synergistic at all three of the fixed combination ratios. In our mouse model of PAM, a combination of amphotericin B (2.5 mg/kg of body weight) and azithromycin (25 mg/kg) protected 100% of the mice, whereas amphotericin B alone (2.5 mg/kg) protected only 27% of mice and azithromycin alone (25 mg/kg) protected 40% of mice. This study indicates that amphotericin B and azithromycin are synergistic against the Lee strain of *N. fowleri*, suggesting that the combined use of these agents may be beneficial in treating PAM.**

Primary amebic meningoencephalitis (PAM) is a rapidly fatal infection caused by the free-living ameba *Naegleria fowleri*. Victims are usually healthy young persons with a recent history of swimming or other water exposure. The ameba enters the nasal cavity during inhalation or aspiration of contaminated water and then migrates along the olfactory nerves, crosses the cribriform plate, and enters the central nervous system. Once in the brain, *N. fowleri* causes extensive inflammation, hemorrhage, and necrosis, leading to death in 3 to 7 days (15).

The mortality of persons with PAM is >95% (3) owing to the rapid progression of the disease, the often delayed diagnosis, and the lack of effective therapeutic agents. A wide range of antiparasitic, antimicrobial, and other pharmacologic agents have been evaluated against *N. fowleri*, but most of these agents have shown limited activity against the protozoon (5, 7–9, 11, 26, 28, 29). Amphotericin B is the only agent with established clinical efficacy for PAM, and at least eight persons have been treated successfully with amphotericin B alone or in combination with other drugs (1, 2, 12, 17, 20, 22, 30, 31). However, not all patients treated with amphotericin B either alone or in combination have survived PAM (6, 23–25). Moreover, amphotericin B is one of the most toxic antibiotics used today, and it may cause serious renal toxicity and electrolyte disturbances as well as hematopoietic effects and damage to other organs.

We previously investigated the activities of novel agents against *N. fowleri* in vitro and in a mouse model of PAM in order to identify agents with potential clinical usefulness against this infection. In these studies, we found that azithromycin was highly active against *N. fowleri* in vitro and that it protected 100% of mice infected with *N. fowleri* at a dose of 75 mg/kg of body weight/day for 5 days (10). In contrast, amphotericin B protected only 50% of mice at a dose of 7.5 mg/kg/day, whereas all control mice died during the 28-day observation period. Since azithromycin is a relatively nontoxic agent that might be useful in treating PAM alone or in combination with amphotericin B, we evaluated the combined activity of azithromycin and amphotericin B in vitro and in vivo. This report presents the results of our studies with these two agents.

MATERIALS AND METHODS

Amebae and cultivation. The Lee (M67) strain of *N. fowleri* used in this study was originally isolated from a 15-year-old female who died from primary amebic meningoencephalitis in 1968 (15) and has been maintained by 67 passages in mice to retain maximum virulence. The Lee (M67) strain was cultured axenically without agitation in Mix medium (15). Stock cultures of *N. fowleri* were maintained at 37°C in 25-cm^2 polystyrene culture flasks containing 10 ml of Mix medium (15).

Therapeutic agents. Amphotericin B in aqueous solution at 250 μg/ml (Sigma-Aldrich Inc., St. Louis, MO) was diluted in sterile deionized water to obtain the final concentrations used for the in vitro studies. For in vivo studies, amphotericin B powder, consisting of 45% amphotericin B, 35% deoxycholic acid sodium, and 20% sodium phosphate (Sigma-Aldrich Inc.), was dissolved in sterile deionized water to provide the final concentrations needed to administer the indicated dosages to mice. Azithromycin for injection (Zithromax; Pfizer Inc., New York, NY), consisting of powdered azithromycin dehydrate, was dissolved in and diluted with sterile deionized water to provide the concentrations and doses used in this study.

In vitro studies. For each drug study, 30 ml of Mix medium was inoculated with 10^4 amebae/ml from 72-hour stock cultures. A combination of amphotericin B and azithromycin solutions was added to experimental flasks to obtain the required drug concentrations, while control flasks received the same volume of sterile deionized water. The agents were tested in combination at three different concentrations, and each combination was performed in triplicate.

The flasks containing amebae and experimental agent combinations were vortexed, and 10-ml aliquots were distributed to each of three culture flasks and

* Corresponding author. Mailing address: Department of Biomedical Sciences, Oklahoma State University Center for Health Sciences, Tulsa, OK 74107-1898. Phone: (918) 561-8260. Fax: (918) 561-8414. E-mail: shannongoswick@sbcglobal.net.

▽ Published ahead of print on 23 October 2006.

TABLE 1. FIC_{50}s and sums of FIC_{50}s of amphotericin B and azithromycin in combination against *N. fowleri* in vitro

Time of infection and amphotericin B/azithromycin ratio	Amphotericin B		Azithromycin		Sum of FIC_{50}s
	IC_{50} (nM)	FIC_{50}	IC_{50} (nM)	FIC_{50}	
Day 2					
1:3	34.2	0.311	13.9	0.464	0.775
1:1	26.4	0.241	7.1	0.238	0.478
3:1	27.9	0.254	2.6	0.088	0.342
Day 3					
1:3	18.3	0.167	16.4	0.550	0.716
1:1	33.9	0.309	9.2	0.308	0.616
3:1	33.3	0.303	3.1	0.103	0.406

maintained at 37°C. Cell growth was determined daily for a period of 4 days and then again at day 7 with a Coulter counter (model Z_F; Coulter Electronics, Inc., Hialeah, FL). A 200-μl aliquot of each cell suspension was added to 9.8 ml of electrolyte solution containing 0.5% (vol/vol) formalin and 0.4% (wt/vol) NaCl in deionized water. Cuvettes were vortex shaken to separate cell aggregates and then read within 5 min. Four successive counts were obtained for each cuvette. The most deviant count was excluded, the mean of the remaining nine counts (three flasks, with three counts each) was determined, and ameba growth was expressed as the number of amebae per milliliter.

Initially, the concentration of each agent that inhibited 50% of *N. fowleri* growth when used alone (50% inhibitory concentration [IC_{50}]) was determined by fitting a nonlinear regression curve to the concentration-percent inhibition growth curve (Graphpad Prism, San Diego, CA). Next, the inhibitory effects of amphotericin B and azithromycin combinations were determined at fixed concentration ratios of 3:1, 1:1, and 1:3, such that 100% drug combinations contained 75% amphotericin B at its IC_{50} and 25% azithromycin at its IC_{50}, 50% amphotericin B at its IC_{50} and 50% azithromycin at its IC_{50}, and 25% amphotericin B at its IC_{50} and 75% azithromycin at its IC_{50}, respectively. In these studies, six dilutions of each ratio were employed, ranging from 20% to 120% of the fixed-ratio concentrations, in order to construct drug concentration-percent inhibition growth curves and to determine the IC_{50} of each drug in each fixed-ratio combination. The fractional IC_{50} (FIC_{50}) of each drug was then calculated as the IC_{50} of the drug in combination divided by the IC_{50} of the drug alone. The FIC_{50}s of amphotericin B and azithromycin at the different concentration ratios were used to plot isobolograms. The sum of the FIC_{50}s of amphotericin B and azithromycin for each fixed-ratio combination determines whether the combined effect of the drugs is additive, synergistic, or antagonistic. A sum of 1.0 represents an additive effect, a sum of <1.0 represents a synergistic effect, and a sum of >1.0 represents an antagonistic effect (4).

Cell harvesting and inoculation for in vivo studies. Amebae were harvested for mouse inoculations after 72 h of culture in Mix medium at 37°C. The amebae were centrifuged at 2,000 × *g* for 10 min, washed, and resuspended in Page saline to provide a final concentration of 2×10^6 amebae/ml. Male 21-day-old CD-1 mice weighing approximately 24 g (Charles River Laboratories Inc., Wilmington, MA) were housed in plastic cages and given free access to food and water. Mice were inoculated by intranasal instillation of 10 μl Page saline containing 2×10^4 amebae into a single nare under isoflurane anesthesia (Aerrane; Baxter Caribe Inc., Deerfield, IL). All animal studies were conducted in accordance with standard animal experimentation guidelines and with the approval of the Animal Care and Use Committee at the Oklahoma State University Center for Health Sciences.

Treatment of experimental amebic meningoencephalitis. Experimental drug treatments began 72 h after inoculation of amebae and continued once daily for a period of 5 days. Mice were randomly divided into four groups of 10, with each group receiving a different treatment. The control group received intraperitoneal injections of 0.9% sodium chloride (Abbott Laboratories, Chicago, IL). The treatment groups received intraperitoneal injections of 2.5 mg/kg amphotericin B alone, 25 mg/kg azithromycin alone, or a combination of 2.5 mg/kg amphotericin B and 25 mg/kg azithromycin. The in vivo studies were conducted twice.

Mortality and mean time to death. Mice were held for 28 days postinoculation, and the cumulative percent dead was recorded on a daily basis. The mean time to death was also determined for each treatment group. Percent protection was calculated using the expression $100 - (x/y) \times 100$, where x is the percentage of treated mice that died and y is the percentage of control mice that died (14). To verify the cause of death, brain tissue was cultured for amebae from dead or moribund mice. Amebae were observed microscopically in cultures obtained from brain tissues of all infected mice that died during the 28-day observation period.

RESULTS

In vitro studies. The activity of combined amphotericin B and azithromycin on the growth of the Lee (M67) strain of *N. fowleri* is reported in Table 1 and Fig. 1. Table 1 lists the IC_{50}s and the FIC_{50}s of each agent when combined in 1:3, 1:1, and 3:1 fixed-concentration ratios of amphotericin B to azithromycin. The sum of FIC_{50}s was <1 for all three fixed-concentration ratios on both days, indicating that amphotericin B and azithromycin have synergistic activity against *N. fowleri* at all concentration ratios tested. The 1:3 amphotericin B-to-azithromycin ratio resulted in a higher sum of FIC_{50}s than did the 1:1 ratio, and the 3:1 ratio produced the lowest sum of FIC_{50}s, suggesting that greater synergy may occur with this concentration ratio, while less synergy is obtained with the 1:3 concentration ratio.

Figure 1 shows an isobologram depicting the relationship between the FIC_{50}s of azithromycin and amphotericin B for the three fixed-concentration ratios on days 2 and 3 of incubation. The FIC_{50}s of amphotericin B were similar at all fixed-ratio concentrations, whereas the FIC_{50}s of azithromycin varied considerably and were highest with the 1:3 amphotericin B-to-azithromycin ratio and lowest with the 3:1 ratio on both days 2 and 3 of incubation. All of the data points are below the straight line of additivity, indicating that amphotericin B and azithromycin exerted a synergistic effect against *N. fowleri* at all fixed-concentration ratios tested.

In vivo studies. The activity of combined amphotericin B and azithromycin was also evaluated for its potential therapeutic effectiveness in a mouse model of primary amebic meningoencephalitis (18). The doses employed in this study were

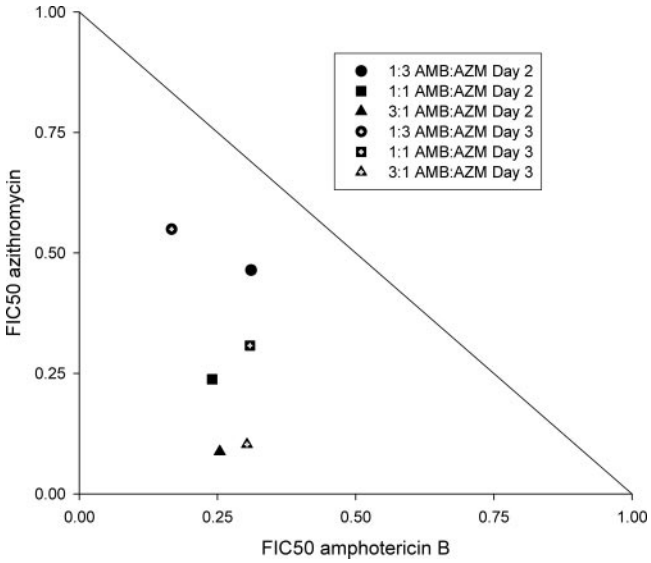

FIG. 1. Isobologram of the interaction of amphotericin B (AMB) and azithromycin (AZM) against the Lee (M67) strain of *N. fowleri*. Each point represents the FIC_{50} of these agents on day 2 or 3 of incubation.

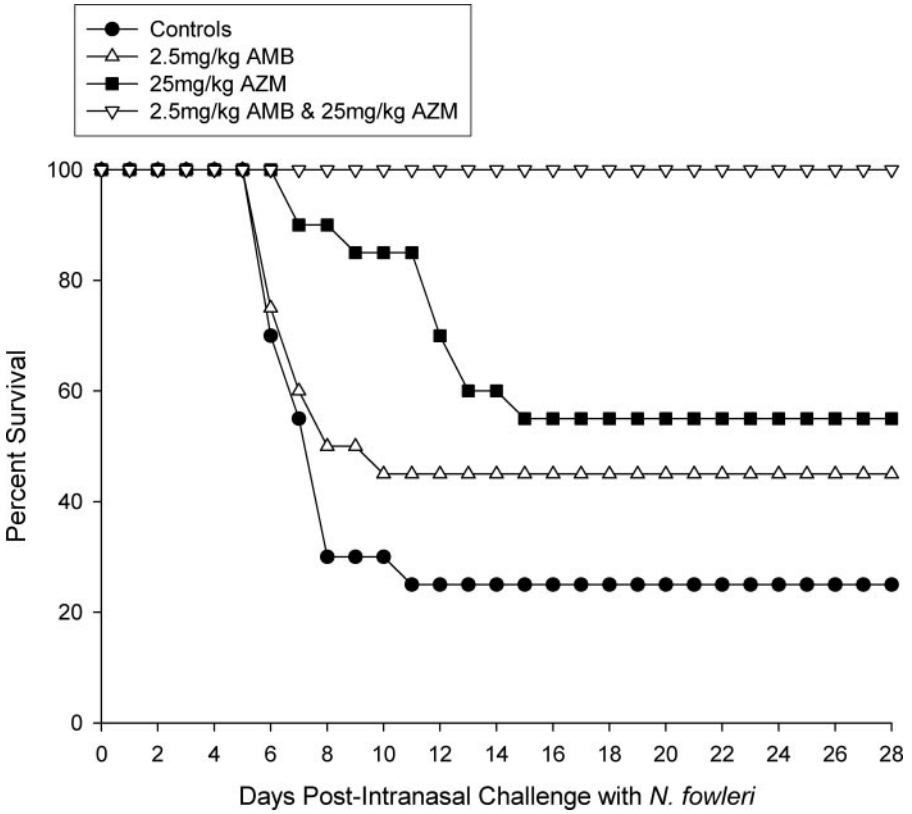

FIG. 2. Survival of mice after inoculation with *N. fowleri* followed by treatment with amphotericin B (AMB), azithromycin (AZM), or a combination of both injected once daily on days 4 to 8 after inoculation. Control mice received sterile saline.

based on our previous in vivo studies with each agent used alone (10). In the present study, we used doses of amphotericin B and azithromycin that had previously been shown to protect 30% and 40% of animals inoculated with *N. fowleri*, respectively. The in vivo experiments were conducted twice, with 10 mice per treatment group, and the results were combined (Fig. 2). One mouse in the combination treatment group died due to other causes. This mouse showed no signs of PAM infection prior to death, and *N. fowleri* could not be cultured from the brain tissue. Therefore, the mouse was excluded from the study.

Table 2 shows the numbers of mice, percentages of mortality, mean times to death, and percentages of protection obtained with control saline injections and with each agent alone and in combination. In this study, not all of the control mice died after inoculation with *N. fowleri*; thus, the percent protection is used to describe the treatment effects. Amphotericin B alone at a dose of 2.5 mg/kg/day protected 27% of the mice, with a mean time to death of 7 days, whereas azithromycin alone at 25 mg/kg/day protected 40% of the mice, with a mean time to death of 12.4 days. The mean time to death of azithromycin-treated animals was significantly greater than that of controls, whereas the mean time to death of amphotericin B-treated animals was not significantly different from that of controls ($P < 0.01$). These results are very similar to those of our previous in vivo studies with each agent used alone at the same doses (10). Additionally, the mean time to death of azithromycin-treated animals was significantly greater than that of the amphotericin B-treated animals ($P < 0.01$). The combination of 2.5 mg/kg amphotericin B and 25 mg/kg azithromycin protected 100% of the mice throughout the 28-day observation period. Because there were no deaths due to *N. fowleri* in the combination group, we could not perform the Mann-Whitney U test to test significance. Since the percent protection provided by the drug combination is greater than the sum of the percentages of protection obtained with each agent alone, the in vivo data are consistent with the synergistic effect demonstrated in the in vitro studies described above.

TABLE 2. Percent mortality, mean time to death, and percent protection of mice inoculated with *N. fowleri* and treated for 5 days with amphotericin B, azithromycin, or the combination of both[a]

Treatment group (treatment dose [mg/kg/day])[a]	No. of mice	% Mortality	Mean time to death (days)[c]	% Protection
Control	20	75	7.1	
AMB (2.5)	20	55	7*	27
AZM (25)	20	45	12.4**	40
Combination (2.5 [AMB] and 25 [AZM])	19[b]	0	None died	100

[a] AMB, amphotericin B; AZM, azithromycin.

[b] One mouse died due to other causes. *N. fowleri* could not be cultured from the brain tissue, and the mouse did not exhibit signs of PAM infection prior to death.

[c] *, not significantly different from controls ($P < 0.01$), using Mann-Whitney U test; **, significantly different from controls and amphotericin B treatment group ($P < 0.01$), using Mann-Whitney U test.

DISCUSSION

Synergy occurs when the effect of two agents used in combination is greater than the sum of the individual effects of each agent given alone. The greater the synergy between two agents, the less of each agent is required to produce a specific effect. Synergy may enable the use of lower doses to produce a defined level of efficacy, thereby increasing safety and tolerability. Synergy may also enable two agents, both of which are <50% efficacious, to be combined to produce 100% efficacy. Synergy is demonstrated in vitro by determining the FIC_{50}s of each agent used in combination. If the sum of the FIC_{50}s is 1, then the two agents are additive when used together (19). If the sum is <1, the two agents are synergistic, and if the sum is >1, the two agents are antagonistic (19).

We previously showed that azithromycin is active against *N. fowleri* in vitro and that a dose of 75 mg/kg protected 100% of mice infected with this organism, whereas amphotericin B alone protected only 50% of animals (10). In the present study, we found that the combination of amphotericin B and azithromycin acted synergistically against *N. fowleri* in vitro when these agents were combined in three fixed-concentration ratios, i.e., 1:1, 1:3, and 3:1. The sum of the FIC_{50}s was lowest for the 3:1 amphotericin B-to-azithromycin ratio on both days 2 and 3 of incubation, indicating that this ratio resulted in the greatest degree of synergy of the concentrations tested. The FIC_{50}s of azithromycin were also lowest with the 3:1 ratio, whereas the FIC_{50}s of amphotericin B followed no discernible pattern. These data suggest that the synergistic effect of these two agents may partly result from the potentiation of azithromycin by amphotericin B, thereby leading to lower FIC_{50}s for azithromycin as the proportion of amphotericin B in the combinations increased.

We also determined the combined effect of amphotericin B and azithromycin in a mouse model of PAM. We found that a combination of 2.5 mg/kg amphotericin B and 25 mg/kg azithromycin given once daily for 5 days protected 100% of mice inoculated with *N. fowleri*, whereas these agents protected 27% and 40% of mice, respectively, when used alone. Because the combined use of these agents achieved 100% efficacy, whereas each agent alone produced <50% efficacy, these data are consistent with the synergy demonstrated with these agents in vitro.

Amphotericin B is the only agent with established clinical efficacy in treating human *N. fowleri* infections and has been used alone and in combination with rifampin, fluconazole, sulfadiazine, miconazole, sulfisoxazole, ketoconazole, dexamethasone, ornidazole, and chloramphenicol to successfully treat PAM (1, 2, 12, 17, 20, 22, 30, 31). Seidel et al. (22) showed that amphotericin B had additive or synergistic activity with miconazole against the strain isolated from the patient successfully treated with this combination. However, none of the other agents used in treating PAM survivors has been shown to be synergistic.

Amphotericin B was reported to be synergistic with minocycline and tetracycline when synergy was defined as a fourfold decrease in the MIC of each drug used in combination compared to that for the use of each drug alone (16). The efficacy of amphotericin B was also potentiated by tetracycline in a mouse model of PAM (27). However, the successful treatment of PAM with amphotericin B combined with tetracycline or minocycline has not been reported.

There is little known about the mechanism of action of azithromycin against *N. fowleri*. Azithromycin inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit and blocking peptide bond formation and translocation. Azithromycin has been shown to be widely distributed in brain tissue following systemic administration in humans, whereas amphotericin B exhibits poor penetration of the blood-brain barrier (13). *N. fowleri* exposed to amphotericin B rounds up and fails to form pseudopodia. The ultrastructural abnormalities included alteration of nuclear shape, degeneration of mitochondria, and the appearance of autophagic vacuoles (21).

The present study indicates that azithromycin and amphotericin B are synergistic against the Lee strain of *N. fowleri*, suggesting that the combined use of these agents might provide a useful regimen for treating human infections with this organism. Further studies to determine the lower range of synergistic activity of these two agents should be conducted. Finally, additional synergy studies with other agents could be conducted in order to improve drug selection and treatment of *N. fowleri* infection.

ACKNOWLEDGMENT

We gratefully acknowledge David T. John for providing helpful advice and laboratory facilities.

REFERENCES

1. **Anderson, K., and A. Jamieson.** 1972. Primary amoebic meningoencephalitis. Lancet **i:**902–903.
2. **Apley, J., S. K. Clarke, A. P. Roome, S. A. Sandry, G. Saygi, B. Silk, and D. C. Warhurst.** 1970. Primary amoebic meningoencephalitis in Britain. Br. Med. J. **1:**596–599.
3. **Barnett, N. D., A. M. Kalpan, R. J. Hopkin, M. A. Saubolle, and M. F. Rudinsky.** 1996. Primary amoebic meningoencephalitis with *Naegleria fowleri*: clinical review. Pediatr. Neurol. **15:**230–234.
4. **Berenbaum, M. C.** 1978. A method of testing synergy with any number of agents. J. Infect. Dis. **137:**122–130.
5. **Carter, R. F.** 1969. Sensitivity to amphotericin B of a *Naegleria* sp. isolated from a case of primary amoebic meningoencephalitis. J. Clin. Pathol. **22:** 470–474.
6. **Cursons, R., J. Sleigh, D. Hood, and D. Pullon.** 2003. A case of primary amoebic meningoencephalitis: North Island, New Zealand. N. Z. Med. J. **116:**U712.
7. **Dhu, P.** 1982. Effect of antimalarial drugs on *Naegleria fowleri*. Med. J. Aust. **1:**13.
8. **Donald, J. J., E. A. Keys, R. T. Cursons, and T. J. Brown.** 1979. Chemotherapy of primary amoebic meningoencephalitis (primary amebic meningoencephalitis). N. Z. J. Med. Lab. Technol. **1979**(March)**:**23–26.
9. **Duma, R. J., and R. Finley.** 1976. In vitro susceptibility of pathogenic *Naegleria* and *Acanthamoeba* species to a variety of therapeutic agents. Antimicrob. Agents Chemother. **10:**370–376.
10. **Goswick, S. M., and G. M. Brenner.** 2003. Activities of azithromycin and amphotericin B against *Naegleria fowleri* in vitro and in a mouse model of primary amebic meningoencephalitis. Antimicrob. Agents Chemother. **47:** 524–528.
11. **Goswick, S. M., and G. M. Brenner.** 2003. Activities of therapeutic agents against *Naegleria fowleri* in vitro and in a mouse model of primary amebic meningoencephalitis. J. Parasitol. **89:**837–842.
12. **Jain, R. R., S. S. Prabhakar, M. M. Modi, R. R. Bhatia, and R. R. Sehgal.** 2002. *Naegleria* meningitis: a rare survival. Neurol. India **50:**470–472.
13. **Jaruratanasirikul, S., R. Hortiwakul, T. Tantisarasart, N. Pheunpathom, and S. Tussanasunthornwong.** 1996. Distribution of azithromycin into brain tissue, cerebrospinal fluid, and aqueous humor of the eye. Antimicrob. Agents Chemother. **40:**825–826.
14. **John, D. T., R. R. Weik, and A. C. Adams.** 1977. Immunization of mice against *Naegleria fowleri* infection. Infect. Immun. **16:**817–820.
15. **John, D. T.** 1993. Opportunistically pathogenic free-living amebae, p. 143–246. *In* J. P. Kreier and J. R. Baker (ed.), Parasitic protozoa, 2nd ed., vol. 3. Academic Press Inc., San Diego, CA.
16. **Lee, K. K., S. L. Karr, M. M. Wong, and P. D. Hoeprich.** 1979. In vitro susceptibilities of *Naegleria fowleri* strain HB-1 to selected antimicrobial

agents, singly and in combination. Antimicrob. Agents Chemother. **16:**217–220.
17. **Loschiavo, F., T. Ventura-Spagnolo, E. Sessa, and P. Bramanti.** 1993. Acute primary meningoencephalitis from entamoeba *Naegleria fowleri*. Acta Neurol. (Napoli) **15:**333–340.
18. **Martinez, A. J., E. C. Nelson, and R. J. Duma.** 1973. Animal model: primary amebic (*Naegleria*) meningoencephalitis in mice. Am. J. Pathol. **73:**545–548.
19. **Ohrt, C., G. D. Willingmyre, P. Lee, C. Knirsch, and W. Milhous.** 2002. Assessment of azithromycin in combination with other antimalarial drugs against *Plasmodium falciparum* in vitro. Antimicrob. Agents Chemother. **46:**2518–2524.
20. **Poungvarin, N., and P. Jariya.** 1991. The fifth nonlethal case of primary amoebic meningoencephalitis. J. Med. Assoc. Thai. **74:**112–115.
21. **Schuster, F. L., and E. Rechthand.** 1975. In vitro effects of amphotericin B on growth and ultrastructure of the ameboflagellates *Naegleria gruberi* and *Naegleria fowleri*. Antimicrob. Agents Chemother. **8:**591–605.
22. **Seidel, J. S., P. Harmatz, G. S. Visvesvera, A. Cohen, J. Edwards, and J. Turner.** 1982. Successful treatment of primary amoebic meningoencephalitis. N. Engl. J. Med. **306:**346–348.
23. **Shenoy, S., G. Wilson, H. V. Prashanth, K. Vidalakshmi, B. Dhanashree, and R. Barath.** 2002. Primary meningoencephalitis by *Naegleria fowleri*: first reported case from Mangalore, South India. J. Clin. Microbiol. **40:**309–310.
24. **Sithinamsuwan, P., T. Sangruchi, P. Chiewvit, and N. Poungvarin.** 2001. Free-living ameba infections of the central nervous system in Thailand: report of two patients and review of the literature. Intern. Med. J. Thai. **17:**350–360.
25. **Stevens, A. R., S. T. Shulman, T. A. Lansen, M. J. Cichon, and E. Willaert.** 1981. Primary amoebic meningoencephalitis: a report of two cases and antibiotic and immunologic studies. J. Infect. Dis. **143:**193–199.
26. **Thong, Y. H., B. Rowan-Kelly, C. Shepard, and A. Ferrante.** 1977. Growth inhibition of Naegleria fowleri by tetracycline, rifamycin, and miconazole. Lancet **22:**876.
27. **Thong, Y. H., B. Rowan-Kelly, A. Ferrante, and C. Shepard.** 1978. Synergism between tetracycline and amphotericin B in experimental amoebic meningoencephalitis. Med. J. Aust. **1:**663–664.
28. **Thong, Y. H., B. Rowan-Kelly, and A. Ferrante.** 1979. Delayed treatment of experimental amoebic meningo-encephalitis with amphotericin B and tetracycline. Trans. R. Soc. Trop. Med. Hyg. **73:**336–337.
29. **Thong, Y. H., B. Rowan-Kelly, and A. Ferrante.** 1979. Treatment of experimental *Naegleria* meningoencephalitis with a combination of amphotericin B and rifamycin. Scand. J. Infect. Dis. **11:**151–153.
30. **Vargas-Zepeda, J., A. V. Gomez-Alcala, J. A. Vazquez-Morales, L. Licea-Amaya, J. F. Jonckheere, and F. Lares-Villa.** 2005. Successful treatment of *Naegleria fowleri* meningoencephalitis by using intravenous amphotericin B, fluconazole, and rifampicin. Arch. Med. Res. **36:**83–86.
31. **Wang, A., R. Kay, W. S. Poon, and H. K. Ng.** 1993. Successful treatment of amoebic meningoencephalitis in a Chinese living in Hong Kong. Clin. Neurol. Neurosurg. **95:**249–252.